

Protective effect of Silymarin against kidney injury induced by carbon tetrachloride in male rats

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(Received May 18, 2018; Accepted June 26, 2018)

Abstract

The herbal drugs have a protective effect for kidney function against chemical toxicity. 24 male rats divided into 4 groups and treated as following, control group administrated orally with 1ml/kg. B.W physiological solution (0.9%), One dose Carbon Tetrachloride (CCl₄) 3 ml/kg. B.W, Silymarin 150 mg/kg. B.W and Silymarin 150 mg/kg. B.W with CCl₄ 3 ml/kg. B.W for 30 days. Oxidative stress resulted by CCl₄ caused increasing in Creatinine, Urea, total protein, Albumin, malondialdehyde (MDA) levels decreasing in Glutathione (GSH) and superoxide dismutase (SOD) levels in serum and congestion, degeneration and desquamation in kidney tissue. We concluded that Silymarin showed protective effect via increasing GSH, decreasing creatinine, Urea, total protein and MDA levels in serum and protect kidney tissue in rats.

Keywords: Silymarin, CCl₄, Kidney toxicity, Antioxidant
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التأثير الوقائي للسلمارين ضد اصابة الكلى الناجم عن رباعي كلوريد الكربون في ذكور الجرذان

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الخلاصة

تمتلك العقاقير الطبية تأثيراً وقائياً لوظائف الكلى ضد سمية المواد الكيميائية. تم تقسيم ٢٤ من ذكور الجرذان إلى أربع مجموعات وعوملت كالاتي، مجموعة السيطرة تم معاملةتها فمويا بالمحلول الملحي الفسلجي (٠,٩%)، مجموعة CCl₄ أخذت جرعة مفردة من مادة رباعي كلوريد الكربون (٣ مل/كغم وزن جسم)، مجموعة السلمارين (١٥٠ ملغم/كغم وزن جسم) ومجموعة السلمارين مع CCl₄ ولمدة ثلاثين يوماً. الجهد التأكسدي الناجم عن CCl₄ تسبب بزيادة في مستوى الكرياتينين، اليوريا، البروتين الكلي، الألبومين، المالوندايديهايد وانخفاضاً في مستوى الكلوتاثيون والسوبر اوكسيد دسيموتيز في مصل الدم واحتقان دموي وتحطم وتوسف في نسيج الكلى بينما اظهر السلمارين تأثيراً وقائياً من خلال زيادة مستوى الكلوتاثيون والالبومين وخفض الكرياتينين واليوريا والبروتين الكلي والمالوندايديهايد في مصل الدم وحماية نسيج الكلى في الجرذان.

Introduction

Kidney is a vital organ to achieve homeostasis and has important biological function in regulation of extracellular fluid volume, acid-base balance, electrolytes and excretion of metabolic wastes (1). Kidney is very sensitive to xenobiotic (2,3). Xenobiotic such as chemotherapeutic materials and bacteria, viruses' metabolites usually produce

reactive oxygen species ROS and or reactive nitrogen species RNS during xenobiotic metabolism (4,5). ROS and RNS have been demonstrated cause cell intoxication when they generate in excessive amount or depletion of antioxidant defense systems which cause lipid peroxidation and oxidative stress (6,7). Carbone tetrachloride (CCl₄) intoxication chemical compound has been used as experimental model to induce stress mimics

pathophysiological status in vivo (8). CCl_4 generate free radicals in tissue in addition CCl_4 toxicity represented in formation of carbon trichloride (CCl_3) which interact with oxygen to form CCl_3O_2 (8). Herbal drugs used in widespread around the world because their activity against many diseases and intoxication (9). Silymarin is a compound derived from *Silybum marianum* is an ancient medicinal plant the common name is milk thistle. silymarin is the active compound which containing four flavonolignan isomers: silybin, isosilybin, silydianin and silychristin. silybin is the most active compound (9-11). Silymarin and one of its component silybin are available now as capsules and tablets under trade names such as legalon, livergol and silipide (9). Silymarin is a safe herbal compound soon it used according to the physiological recommended doses (12,13) it has been used as antifibrotic, antioxidant, anti-inflammatory and against hepatotoxicity. (12,14,15). The mechanism of silymarin action is still poorly understood (16). Data from literatures indicated that silymarin act as antioxidant, free radical scavengers, regulator of intracellular glutathione GSH and stabilizing cell membrane (17-19). This study aimed to detect the biological vitality of silymarin in protecting kidney tissue against oxidative stress induced by CCl_4 toxicity.

Materials and methods

Experimental design

Twenty-four adult male rats weighted 200-250 g housed at the animal units in faculty of veterinary / Tikrit university. Animals were free access to standard rat food, water and standard environmental conditions in the animal house unit. during the experimental period that continued for 30 days. Rats divided equally into four groups first is control group treated orally with physiological solution (0.9%) while the other three groups treated orally with single dose of CCl_4 (3ml/kg) added to equivalent volume of Olive oil, orally dose of silymarin (150mg/kg) (19) and silymarin (150mg/kg) with CCl_4 (3ml/kg) respectively. Silymarin were supplied from Signova Pharma Pvt Ltd company.

Serum preparation

Rats anesthetized and sacrificed, blood samples were taken through cutting the Jugular vein. The blood serum collected using centrifuge (3000 rpm for 15 min) and kept at $-20\text{ }^\circ\text{C}$. To speculate renal function of experimental animals treated by CCl_4 and silymarin.

Biochemical analysis

Urea, Creatinine, total protein and Albumine levels in serum were measured, and also to clarify the association of renal function and oxidative stress, MDA, GSH and SOD were measured. All serum analyzing used by

spectrophotometer and analysis kits materials and methods that obtained from supplier company BIOLABO SAS (20).

Histological study

Kidney tissue were obtained to evaluate whether CCl_4 and silymarin elicit alteration in the kidney tissue. Tissue immediately fixed in 10% formalin for 24 hours then washed via water and dehydrated by gradient series concentration of alcohol, embedded in paraffin and slices of 5 μm thickness by microtome, sections stained with Hematoxylin and Eosin (HE) (21). Slides were observed using light microscopy for diagnosis the kidney histology and morphology.

Statistical Analysis

Data analyzed using one-way analysis of variance (one-way ANOVA) independent samples test. Differences between groups determined by Duncan multiple range test. The value $P<0.01$ regarded as statistically significant value (22).

Results

Serum analyzing results (Table 1 and 2) demonstrated the effect of CCl_4 to increase levels of creatinine, urea, MDA and decreasing in total protein, albumin, GSH and SOD in compare to control group. Silymarin group showed significant decrease in creatinine, urea and MDA and significant increase in albumin and GSH but no differences noticed in total protein, albumin and SOD in compare to control group. While treatment by CCl_4 with silymarin showed decreasing in creatinine, Urea, MDA levels and increasing in Total protein, albumin and GSH in compare to CCl_4 group, but no difference in SOD level. Kidney tissue demonstrated the damage effect of CCl_4 represented in degeneration in glomerular, tubular and interstitial tissue, desquamation, necrosis in some cells and congestion. (Figure 1-B) in compare to control group (Figure 1-A). While silymarin showed decreasing in degeneration, desquamation and congestion (Figure 1C, 1D) in compare to CCl_4 group (Figure 1-B). Creatinine and Urea are metabolites usually used for determination of glomerular filtration rate and then renal function (23). CCl_4 caused an impairment to clear the blood from these metabolites into the urine through damages in kidney tissue studies reported that CCl_4 Initiated lipid peroxidation, reduces renal (GSH/GSSG) ratio in kidney cortex, microsomes and mitochondria (24,25) which reflect the high plasma levels of creatinine and urea (Table 1, Figure1-B), this diagnostic as impaired renal function occurred as a result to renal injury in rats (26,27). Rats administrated with CCl_4 showed high oxidative stress (Table 1) and damages in kidney tissue (Figure 1-B).

Table 1: Assessment of kidney function through levels of some biochemical parameters

Parameters	Groups			
	Control	CCL4 (3 ml/kg.B.W)	Silymarin (150 mg/kg.B.w)	CCL ₄ + Silymarin
Creatinine (mg/dl)	0.8333±0.081 ^b	1.1333±0.136 ^a	0.7±0.089 ^b	0.85±0.447 ^b
Urea (mg/dl)	40.916±1.696 ^c	51.266±1.291 ^a	38.75±0.967 ^c	46.433±0.852 ^b
Total protein(g/dl)	7.266±0.175 ^b	5.8±0.368 ^d	6.65±0.339 ^c	8±0.303 ^a
Albumin(g/dl)	4.316±0.306 ^a	3.55±0.251 ^b	4.65±0.327 ^a	4.533±0.250 ^a

Values expressed means (±SEM) of 6 rats. Different letters mean significant difference at P<0.01.

Table 2: Changes in oxidant- antioxidant levels in experimental groups

Parameters	Groups			
	Control	CCL4 (3 ml/kg.B.W)	Silymarin (150 mg/kg.B.w)	CCL ₄ + Silymarin
Glutathione(μmol/L)	4.098±0.442 ^{a,b}	2.235±0.326 ^c	4.633±0.338 ^a	3.616±0.231 ^b
Superoxide dismutase (μmol/L)	1.183±0.124 ^a	0.953±0.029 ^b	0.988±0.162 ^{a,b}	0.973±0.129 ^b
Malondialdehyde (μmol/L)	1.966±0.196 ^c	4.2±0.296 ^a	1.58±0.263 ^c	2.771±0.607 ^b

Values expressed means (±SEM) of 6 rats. Different letters mean significant difference at P<0.01.

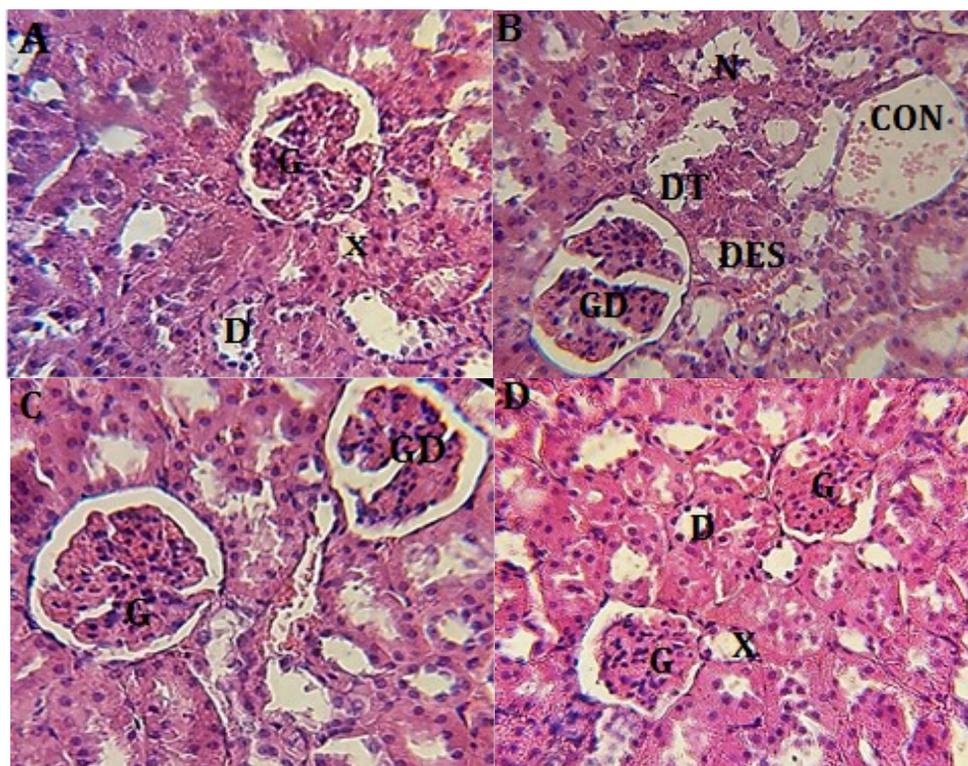


Figure 1: A. Control group showing normal kidney tissue glomeruli (G), proximal (X) and distal tubular (D). B. Damage in kidney tissue resulted via CCl₄, glomerular degeneration (GD), degeneration in renal tubular (DT), interstitial tissue, desquamation of some cells (DES), congestion (CON) and moderate to severe necrosis (N). C and D Show effectiveness of Silymarin in attenuated CCl₄ effect in compare to CCl₄ group. (H&E, 40x).

Discussion

The metabolic conversion of CCl₄ by cytochrome p-450 generates reactive free radicals that initiate cell necrosis,

lipid peroxidation, elevate MDA levels, depletion in GSH levels and impair antioxidants activity (28) which affect negatively renal function by exhibit renal vasoconstriction

or inhibit the glomerular filtration coefficient; and decreasing filtration rate (25).

Silymarin has an antioxidant, free radical scavengers, intracellular glutathione GSH regulator and stabilizing cell membrane activity. Pathological changes in kidney tissue has been demonstrated when rats exposed to CCl₄. Animals have unique system for protecting against ROS (29). Rats can resist the elevating in ROS using SOD and GSH. GSH is essential non-enzymatic antioxidant associated with glutathione transferase in attenuated and scavenging free radicals (30) SOD is an enzymatic antioxidants dismutation superoxide to nontoxic molecules (31). In this study Silymarin demonstrated the ability to prevent CCl₄ from lipid peroxidation in kidney tissue and has a cytoprotection activities, also elevated ROS scavengers such as SOD and GSH, and reduced lipid peroxidation which indicated by MDA level. Studies reported (9,26) that silymarin increase gene expression of antioxidant enzymes. Oxidative stress inhibits insulin secretion which making cell to depend on proteins and fats oxidation as an energy source and that cause elevating in urea and creatinine levels. Silymarin has ability to recover pancreatic function to secrete insulin (32). These findings demonstrated that silymarin has a protective role against CCl₄ induced kidney injury by lipid peroxidation.

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